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THE VALUE OF THE COMPLEMENT FIXATION TEST IN GONORRHŒA

A STUDY OF FIVE THOUSAND TESTS

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INTRODUCTION

THIS paper reviews the results of tests on more than 5,000 sera from unselected patients, some suffering from gonorrhœa or syphilis or both, and others with no venereal disease or history of such.

In this country the complement fixation test for gonococcal infection appears to have been practised comparatively little, a neglect which is undeserved, and probably due to the fact that its value as equal to that of the Wassermann reaction has not been recognised. On the other hand, considerable attention has been paid to it on the Continent and in America.

THE HISTORY OF THE TEST

Whilst the methods of complement fixation had been studied in a general manner for some years, it was only after the work of Wassermann and Bruck that the method was applied to the diagnosis of a variety of infections. Muller and Oppenheim [1] were the first (1906) to show that whereas the serum of a patient suffering from gonococcal arthritis proved capable of fixing complement in the presence of an " antigen " in the form of an extract of gonococci, serum from patients suffering from arthritis due to other causes did not cause this fixation of complement.

In the same year (1906) Bruck [2] published a paper showing that rabbits immunised against gonococci, as well as many patients suffering from gonococcal infections, such as salpingitis, urethritis and epididymitis, yielded

sera capable of fixing complement in the presence of an " antigen " consisting of a watery extract of gonococci.

A probable explanation of the failure, or apparent failure, of the test to detect all cases of gonococcal infection was provided by Teague and Torrey [3] (1907), who showed that gonococci could be divided into at least three groups, since certain strains of gonococci, which in complement fixation were strongly specific for their homologous anti-sera, failed to produce complement fixation in the presence of anti-sera from certain heterologous strains.

The *specific* nature of the reaction was supported generally by those workers who investigated the matter— Vannod [4] (1907) and Krumbein and Schatiloff [5] (1908)— showing that the reaction was not given by anti-meningococcal sera with an antigen composed of gonococci, though Wollstein [6] (1907) considered that strict specificity between the meningococcus and gonococcus was lacking. Later, Arkwright [7] (1912) also produced evidence that the meningococcus might provide a serum reacting with gonococcal antigens.

Other papers appeared showing that the complement fixation test was positive with certain cases of gonorrhœa but negative with others, but the number of cases quoted in most papers tended to be small until in 1911 Schwartz and McNeil [8] published considerable observations on the method. These workers confirmed the earlier work, including that of Teague and Torrey, in regard to the multiplicity of strains. The antigens used, therefore, were made from many different sources, apparently in the hope that in this way all strains might be accounted for and represented. The results in over 300 cases showed that the reaction became positive some three to four weeks after infection and tended to disappear six to eight weeks after cure. These authors considered the test to be absolutely specific.

Many other papers were written during the next three years which showed the test to have very distinct possibilities, but in all the failure to react in every case was shown.

The antigen varied with the different workers, most of whom used some type of extract of the gonococcus, and others mere suspensions of the organisms. Van Saun [9] (1912) studied the effects of the culture medium on the

quality of the antigen produced and showed that this factor was not an important one.

Harrison [10] (1914) attempted to make the test more delicate, and in his technique decreased the amount of complement available, increasing the amount of antigen. By this method he obtained similar results with old-standing cases to those obtained by other workers (*i.e.*, a high percentage of positive results), but, unlike others, he also obtained a comparatively high percentage of positive results in the earlier cases.

Thomas and Ivy [11] (1914) published the results of tests cases in 204. The antigen used was an autolysate of gonococci, several strains being found to yield the best antigen. These workers showed that the test was specific, reliable and of great importance in old-standing cases where bacteriological proof is often difficult ; also in " marriage " tests and tests of cure. The desirability of a negative test before marriage is further insisted on by Jones and Simons [12] (1914).

The superiority of a hexavalent over a monovalent antigen was well shown by Ower [13] (1914), who gives the results obtained by testing 130 sera with the two antigens.

Schupe [14] (1916) demonstrated the absence of non-specific positive results in 1,000 tests ; he also found the test to be negative in the earlier stages, positive reactions usually appearing in the fifth to sixth week. Cure was shown to result in a negative reaction within six weeks, those cases which remained positive not being truly cured. In his series of cases arthritis gave 100 per cent. positive results.

Dixon and Priestly [15] (1919), as a result of the study of 625 cases, came to the conclusion that those patients who showed an early strong positive reaction with subsequent fall were among those who did best under treatment.

One of us (T. E. O.,[16] 1922), using a modification of Thomson's technique, published the results of 1,000 tests, showing the high percentage of positive results obtained in cases of active gonorrhœa and the very low incidence of falsely positive results (0·3 per cent.).

Thomson [17] (1923) has investigated the test very fully, elaborating the technique as used by the present authors and approved by the Medical Research Council. This author deals also with the question of superiority of compound antigens compared with those made from

single strains of gonococci, with the mode of preparation of antigens and the results obtained by using the technique he describes.

Zoon [18] (1928), after an extensive study of the test, concludes that as applied by his technique the test is specific, of great diagnostic value except in cases of vulvo-vaginitis of children, and that a syphilitic infection gives no positive reaction in the complement fixation test for gonorrhœa.

Kolmer [19] (1928) considers the test of definite value in the diagnosis of the acute complications of gonorrhœa and in the acute exacerbations of chronic infections; in the detection of latent cases of the disease and in the differential diagnosis of cases of arthritis and pelvic disease in women.

THE TECHNIQUE USED BY THE AUTHORS

This follows the method elaborated by Thomson very closely.

Serum.—This is diluted 1/9 with physiological saline after being inactivated for forty minutes at 55° C. It is important that the inactivation should be carried out within an hour or so of preparing the tests, as otherwise the sera tend to become anti-complementary.

Complement.—Guinea-pig's serum is used and titrated as in No. 1 method, Medical Research Council's Special Report Series, No. 14, for the Wassermann reaction (Harrison's method). Two strengths are used, containing 3 and 4 m.h.d. respectively.

Hæmolytic System.—This is similar to that used for the Wassermann reaction referred to above, *i.e.*, 3 per cent. sheep's cells sensitised with 5 to 6 m.h.d. rabbit *versus* sheep hæmolytic amboceptor.

Antigen.—Consists of an extract of a number of selected strains of gonococci—usually not less than twelve. The gonococcus is grown on Thomson's medium, slightly modified, for twenty-four to forty-eight hours. It is then subcultured and at the end of eighteen to twenty-four hours is washed off with physiological saline. To the suspension thus obtained—usually containing 3,000 to 5,000 millions of organisms per cubic centimetre—is then added the minimum amount of $\frac{N}{5}$ NaOH necessary to

dissolve the gonococci, *i.e.*, to make the suspension clear. The solution is then neutralised with $\frac{N}{5}$ HCl, so as to give a reading of exactly *p*H 7·0. The antigen prepared from each strain is separately tested. It is titrated for anti-complementary activity, which should be low, and is further put up with several known negative sera under the conditions of the test. Not more than *half* the amount which just fails to inhibit hæmolysis under such conditions is taken as the standard amount. Thus, say the antigen allows complete hæmolysis at a dilution of 1/10 with a known negative serum and 3 m.h.d. of complement, whilst there is only partial hæmolysis at a dilution of 1/7·5, the *provisional* titre is taken as 1/20. The antigen must then be put up with a large number of sera in parallel with a standard compound antigen (containing a number of strains), and if it gives good results it is adopted ; if not, it is discarded. In this way a number of simple antigens are prepared, and when twelve or more have been collected they are pooled, the amount of each being in inverse proportion to its titre ; that is to say, if two antigens give titres of 1/20 and 1/40 respectively, two parts of the former would be taken with one part of the latter. Finally, when a sufficient number of simple antigens have been collected and pooled, the antigen prepared in this way is titrated out in a similar way to the simple ones before being taken into use. The process is an extremely laborious one—but after a trial of many methods has given by far the best results. If kept sterile, the antigen remains good for many months, or even years, without showing any signs of deterioration. It should be stored in the dark in the ice-chest at about 4° to 6° C.

Setting up of the Tests.—The general plan is similar to that described for No. 1 method, Wassermann test. Three tubes are used for each serum. The first acts as a serum control and contains no antigen. The second and third are similar to one another, except that the second contains 3 and the third 4 m.h.d. of complement. Into each of the three tubes is placed one drop (approximately 0·012 c.c.) of the serum to be examined, with a special hand-dropping pipette made by drawing out a piece of quill tubing. One volume of saline (from a mechanical dropper delivering approximately 0·11 c.c. amounts) is

then added to the second and third tubes and 2 to the first tube (to make up for the absence of antigen) ; the amounts of saline and serum have been previously so calculated that the resulting dilution of serum is 1/9. One volume of complement is now added to each tube (according to previous titration), and finally 1 volume of antigen. Known negative and positive sera are included in the batch of tests and two extra control tubes are also included, one containing 2 volumes of saline and 1 of complement (2 m.h.d.), and the other 1 volume of saline, 1 of complement (2 m.h.d.), and 1 of antigen as used in the test proper. The racks are then placed in the ice-chest at about 4° to 6° C. for eighteen hours. At the end of this time the sensitised cells, which had been prepared the previous day, for titrating the complement, are added, 1 volume to each tube, and the racks incubated at 37° C. in a water bath.

TEST PROPER

Tube 1.	Tube 2.	Tube 3.	Complement Control.	Antigen Control.
1 volume diluted serum. 1 volume complement, 3 m.h.d. 1 volume saline.	1 volume diluted serum. 1 volume complement, 3 m.h.d. 1 volume antigen.	1 volume diluted serum. 1 volume complement, 4 m.h.d. 1 volume antigen.	2 volumes saline. 1 volume complement, 2 m.h.d. —	1 volume saline. 1 volume complement, 2 m.h.d. 1 volume antigen.

Ice-chest at 4—6° C. for eighteen hours.
One volume sensitised cells.

No fixed time for reading can be given ; as soon as the negative control and the complement and antigen controls show complete hæmolysis, reading can be begun, provided the serum control in any particular case shows complete hæmolysis. First of all the negative results are noted, and five to ten minutes after this all tests can be read. If the complement is an active one and all controls are clear after five minutes, incubation for a further five minutes is sufficient. If, however, controls go more slowly, it is better to wait for a further five minutes—

fifteen minutes in all ; only experience can decide when a test should be read. If one waits too long one will err on the negative side ; on the other hand, when the controls go slowly, incubation should be more prolonged, and the results of the tests on any sera showing a definite degree of anti-complementary activity should be accepted with reserve. Considerable judgment is necessary in reading the results, and the greater the number of the tests set up in one batch within limits, say, four dozen, the more reliable the results.

The tests were all carried out by the technique which has been described and the results were recorded as being :—

$$++, +\pm \text{ or } + = \text{positive.}$$
$$\pm = \text{doubtful.}$$
$$\text{less than } \pm = \text{negative.}$$

The one plus result indicates complete failure of hæmolysis in the tube containing the lesser amount of complement (3 m.h.d.) and some slight failure of hæmolysis in the other tube also. The plus minus results imply a partial hæmolysis in the tube containing the lesser amount of complement (3 m.h.d.).

Notes on other Methods of Carrying out the Test, with especial Regard to Antigens, Types and Strains of Gonococci.

As in other complement fixation reactions, different workers vary the technique : Schwartz and McNeil employ constant amounts of complement and antigen, varying the amount of patient's serum. Kolmer also uses this method of obtaining a quantitative result. Ower uses constant amounts of complement and of patient's serum and varies the amount of antigen. Thomson uses constant amounts of patient's serum and of antigen, varying the complement. It is this last method which the authors have followed in order to make the test roughly quantitative.

Schwartz and McNeil, Kolmer and Thomson all use the long period of fixation given by some eighteen hours at ice-box temperature before the addition of the hæmolytic system and the final incubation, and the authors have found this practice to be essential if delicate results are

to be obtained. Ower, however, allows fixation for thirty minutes only in a water-bath at a temperature of 37·5° C.

The next most important factor in regard to this test is the antigen ; indeed, this is of the utmost importance, since the amount of detectable antibody produced in gonococcal infections is frequently very small, the success of the test depending very largely on the antigen which shows good combining powers.

Antigens used can be divided into those which are mere suspensions and those which are extracts or autolysates of the organisms.

Those in the first group are capable of fixing only small amounts of complement in the presence of sera, even if these be of high antibody content. Thus the test will, with such antigens, lack the degree of sensitiveness necessary in the diagnosis as well as the required margin for experimental error. Martin [20] used such emulsions of gonococci, but by heating them to 80° C. probably effected some extraction of the organisms and produced an antigen more properly considered as of group 2. Lederer and Hecht,[21] as well as Brahms,[22] used the commercial gonococcal vaccine " Arthigon " as an antigen and found that it was inferior to the autolysate antigens advocated by Teague and Torrey and many other workers. McDonagh and Klein [23] used emulsions of gonococci as their antigen.

Extracts and autolysates of gonococci for use as antigens have been prepared in a large number of ways. Distilled water and heating was the method employed by Teague and Torrey and followed by many other workers. Some have substituted rapid shaking or maceration for several days at room temperature or 37° C. for the action of heat.

Finkelstein and Gershun [24] obtained their extracts by adding antiformin to emulsions of the organisms. Thomson obtains an extract by solution of the gonococci in caustic soda, neutralising the resulting solution with hydrochloric acid.

Warden [25] contends that the fatty and lipoidal portions of the autolysate of gonococci possess more antigenic power than the water-soluble nitrogenous parts. Using alcoholic extracts of the organism this worker obtained a higher percentage of positive reactions in the early stages of the disease, and the positive reactions were given over

a longer period during treatment. It would appear, therefore, that the test is more sensitive when carried out with these alcoholic extracts, but so far as we are aware this work has not been extended or confirmed by other workers except with reference to the test as applied to the diagnosis of tuberculosis.

Whatever the method of preparation of the antigens, advancing knowledge and greater experience of the test have shown very clearly the advantages of the polyvalent over the monovalent preparation.

The factors which govern the production of antigens of great sensitivity, specificity and low anti-complementary activity are little understood. The work of Van Saun is not conclusive. Thomson has shown that with his method of preparing antigens the effectiveness of the preparation is governed by the factors of concentration of the extracted substance, thoroughness of the solution, reaction of the finished product and, finally, of the saline content.

Reference has already been made to the importance of different strains of gonococci in the preparation of successful antigens. Teague and Torrey were the first to show that differences in the gonococci composing them explained why some antigens would react with certain sera only. These workers considered that three strains of gonococci could be assumed. Although the term " strain " had been much employed by various workers, it was not until the work of Atkin [26] (1925) that the question was very fully investigated. Prior to Atkin's work several had divided gonococci into groups by precipitin, absorption, or agglutination reaction or complement fixation, the general tendency being to describe several groups. Atkin divides the organisms into two groups or types only, and shows that there is a possibility of a slow change from one type to the other. In this way no arbitrary number of fixed serological groups can be assumed, and it is evident that the cultures may differ slightly from each other in a greater or lesser degree, depending on the relative numbers from each group or type present in it. Thus it will be seen that the method of employing many cultures to make up a compound antigen for use in the complement fixation test is fully justified, and obviously the only way in which there is any likelihood of all variants being included.

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THE RESULTS OF FIVE THOUSAND TESTS CLASSIFIED AND DISCUSSED.

The results of the tests were classified according to the clinical diagnosis (the authors having seen none of the patients) into the following groups :—

Group 1.—Uncomplicated gonorrhœa, proved by microscopical examination (1,733 cases). For convenience this large group has been subdivided according to the duration of the infection : (*a*) 1 to 10 days ; (*b*) 10 to 21 days ; (*c*) 21 days to 1 year.

Group 2.—Gonorrhœa, proved by microscopical examination, with complications such as epididymitis, prostatitis, arthritis, including ("rheumatism"), iritis, Bartholinitis, Skenitis, peri-urethral abscess and salpingitis (187 cases).

Group 3.—Cases in which the history and clinical signs pointed strongly to a diagnosis of gonorrhœa, but in which gonococci were not found (143 cases).

Group 4.—Cases in which gonorrhœa was suspected, but not proved (207 cases).

Group 5.—Cases in which the evidence against gonococcal infection was unequivocal (2,996 cases).

GROUP 1.—*Uncomplicated Gonorrhœa Proved Microscopically.*
(1,733 Cases.)

	Duration of disease.	Cases.	Positive.	Doubtful.	Negative.	Percentages.			Percentages without doubtfuls.	
						Positive.	Doubtful.	Negative.	Positive.	Negative.
Male Cases	1 to 10 days .	·596	74	63	459	12·4	10·6	77·0	13·9	86·1
	10 to 21 days .	70	32	7	31	45·7	10·0	45·3	50·1	49·9
	21 days to 1 year	317	167	80	70	52·7	25·2	22·1	70·5	29·5
Female Cases	1 to 10 days .	105	4	11	90	3·8	10·5	85·7	4·3	95·7
	10 to 21 days .	67	25	21	21	37·4	31·3	31·3	54·3	45·7
	21 days to 1 year	578	234	154	185	41·1	25·6	32·3	56·4	43·6
Combined Figures	1 to 10 days .	701	78	74	549	11·1	10·6	78·3	12·4	87·6
	10 to 21 days .	137	57	28	52	41·6	20·4	38·0	52·3	47·7
	21 days to 1 year	895	406	234	255	45·4	26·1	28·5	61·4	38·6

FIXATION TEST IN GONORRHŒA

GROUP 2.—*Cases of Gonorrhœa with Complications.*

Result of test.	Males.			Females.			Combined.		
	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.
Positive –	104	87·4	92·8	53	77·9	96·4	157	89·3	94·0
Doubtful .	7	5·9	—	13	19·2	—	20	7·2	—
Negative .	8	6·7	7·2	2	2·9	3·6	10	3·5	6·0

GROUP 3.—*Cases probably Gonococcal in Origin.*

Result of test.	Males.			Females.			Combined.		
	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubfuls.
Positive .	55	48·2	66·3	4	14·5	21·1	59	41·3	57·9
Doubtful .	31	27·2	—	10	34·5	—	41	28·7	—
Negative .	28	24·6	33·7	15	51·0	78·9	43	30·0	42·1

GROUP 4.—*Cases of Doubtful Gonococcal Origin.*

Result of test.	Males.			Females.			Combined.		
	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.
Positive .	16	14·0	25·4	15	14·8	21·8	31	15·0	23·5
Doubtful .	49	43·9	—	26	27·3	—	75	36·2	—
Negative .	47	42·1	74·6	54	57·9	78·2	101	48·8	76·5

GROUP 5.—*Non-gonococcal Cases.*

Result of test.	Males.			Females.			Combined.		
	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.	Cases.	Percentage.	Percentage without doubtfuls.
Positive .	8	0·5	0·6	7	0·4	0·6	15	0·5	0·6
Doubtful .	144	9·0	—	140	10·0	—	284	9·0	—
Negative –	1,443	90·5	99·4	1,254	89·6	99·4	2,697	90·5	99·4

Grand total, 5,266 tests.

From the tables it will be seen that in cases of uncomplicated gonorrhœa neglecting the doubtful reactions :—

(*a*) Up to 10 days   12·4 per cent. gave a positive reaction.
(*b*) 10 to 21 days   52·3 per cent. gave a positive reaction.
(*c*) Over 21 days   61·4 per cent. gave a positive reaction.

That is to say, that of all these cases together, one-half gave positive reactions, or in established cases nearly two out of every three gave such a reaction. This table shows well the increasing proportion of positive reactions with the age of the disease.

When the large number of cases which react poorly from the clinical point of view is considered it is hardly surprising that the positive figures are not higher. The disease is very evidently one associated with the minimum of antibody production, and is therefore not to be considered the happy hunting ground of the serologist anxious for infallible results.

In gonorrhœa, with complications such as epididymitis, arthritis, teno-synovitis, prostatitis, salpingitis, to quote only the more common, the percentage works out at 94 per cent., so that when the diagnosis is in the balance the test in these cases is most valuable.

The difference in the percentage of positive results between Groups 1 and 2 is very well marked. It is almost universally agreed that most complications follow a more or less heavy increase in blood-stream infection, which would account for the increased antibody production resulting in a more strongly positive complement

fixation reaction. The onset of a severe complication such as epididymitis has been frequently noticed to precede immediately a very rapid cure of the disease—a piece of evidence in favour of marked increase of antibodies which succeed in eliminating the gonococcus.

Groups 3 and 4, including, as they do, cases in which the diagnosis was not satisfactorily settled, are not of much help in assessing the value of the test. They merely serve to indicate roughly the percentage of positive and negative reactions which occur in these types of cases.

Group 5 shows a percentage of 99·4 negative results in cases where gonorrhœa could be excluded with reasonable certainty. Since, therefore, the percentage of falsely positive results is as low as 0·6 per cent., the test can claim to rank with any serological reaction for specificity. Such a low percentage of falsely positive results attaches considerable value to the positive results obtained in the cases where the diagnosis was in doubt.

Since the value of a serological reaction must be very greatly diminished if non-specific results are obtained, our figures showing such a low percentage of false positive results are important and bear out what has become abundantly clear with lapse of time, namely, the high degree of specificity possessed by this class of test. Meningococcal infections cannot be entirely ruled out as a possible source of danger, but when the rarity of the condition is considered and its widely different clinical manifestations remembered it will be seen that danger from this source is not worthy of much consideration. It has long been known that the commoner organism, *M. catarrhalis*, can be used to make an antigen in place of gonococci for the purpose of the gonococcal complement fixation test, and that even under such conditions positive results will be obtained in established cases of gonorrhœa. Such results as are obtained in this manner are weak ones in most cases. They would suggest the possibility of infections with the *M. catarrhalis*, providing sera capable of reacting with gonococcal antigens, and thereby giving rise to falsely positive results in the gonococcal complement fixation test. One of us (J. O. O. [27]) has investigated this on behalf of the Medical Research Council, and a paper dealing with the results obtained has recently been published. Briefly, it was found that the danger of such a cross-fixation occurring under conditions

ordinarily met with is not great. That it cannot be positively asserted that the danger is non-existent is shown by the fact that in animal experiments such a cross-fixation can be made to occur very definitely. Further, the results obtained with a few patients show the possibility of this cross-fixation in certain cases. For practical purposes, however, the test may be said to be remarkably specific.

PRACTICAL APPLICATION OF THE TEST WITH ILLUSTRATIONS

Having set out in tabular form the results obtained in a large series of cases and pointed to the evidence of specificity, it would seem to be desirable to examine and discuss certain types of cases, the interpretation of results in general, and at the same time to consider the views of other workers with the test.

There can be little doubt that frequently, both clinically and pathologically, anterior and posterior urethritis are to be considered almost separate conditions ; even those who would deny the truth of a clinical distinction are forced to admit the pathological one.

So long as infection is strictly limited to the anterior urethra the disease is likely to be a localised one ; immediately the posterior urethra is infected there is imminent danger of complications, even of metastatic ones. Certainly there appears to be little production of complement fixing antibody in the case of strictly anterior urethral infection, as shown by the results in most of the very early cases. Very soon after the posterior urethra is infected such antibody is produced in much larger amount and the test becomes positive. Since the infection of the posterior urethra is a variable occurrence in point of time, so is the change from the negative to the positive complement fixation reaction. Our figures show only 14 per cent. positives during the first ten days of the disease, whereas by twenty-one days there are 50 per cent. This figure is increased to only 70 per cent. by the lapse of a further period up to one year, and it is therefore reasonable to consider the period ten to twenty-one days as being the chief period of production of positive results. Such cases as are provoked to reaction before are in most instances infections of the posterior urethra in the male.

In the case of the female the peak in the percentage of positive results is reached at the same time period, ten to twenty-one days, probably as a result of definite infection of the cervix uteri, but more definitely so than is the case with the male, since the percentage does not rise appreciably with the lapse of the further time up to one year (ten to twenty-one days 54 per cent., over twenty-one days 56 per cent.).

It is interesting to compare and contrast the views of other workers.

Dixon and Priestley (1919) considered a strongly positive reaction to be an indication of active gonococcal infection ; that a single negative result was of no value, but that if a second one were obtained at an interval of two to three weeks after the first, this could be taken as strong presumptive evidence against infection. These workers emphasised the value of the positive result in regard to prognosis, stating that cases which were doing well showed a positive result in nine to ten weeks, with subsequent fall, and even expressed the opinion that cases could not be considered to be doing well until the positive result had been obtained.

The administration of ordinary vaccines was found to be almost without effect on the reaction, but detoxicated vaccines, probably by reason of their greater dosage, raised its strength. This artificial raising of the strength of the reaction was found to be without the good import in regard to prognosis that the normally occurring one was.

Walker (1922) [28] is one of the few who consider the fall of the reaction to negative with cure. His cases ceased to react in most instances after three months. Six weeks was sufficient for the disappearance of the reaction in those patients who had been without any kind of complication.

D'Ath, Steenson and Williams (1924), [29] using a similar technique to our own, concluded that patients without gonococcal infection, old or recent, failed to react to the test, and that, exceptionally, patients were met with who were reacting to treatment so well as to go through the course of the disease without ever having reacted to the test. As others had done before them, these workers found that the acute and subacute cases frequently failed to react in the earlier periods. Persistent, strong positive reactions were indicative of con-

tinued infection ; a sudden rise in the strength of the reaction was often associated with the onset of some complication, and the strongest reactions of all were found in cases of metastatic gonococcal infection. As regards fall in the strength of the reaction, these workers considered a gradual decline to indicate a satisfactory progress. Ordinary vaccines in doses up to 250 millions were found to be without effect on the reaction. Lastly, the test must be considered as of great value in the diagnosis of chronic infections.

Zoon (1928) raises the question of whether a positive result very early in the course of a case, and before posterior urethritis is obvious, is of bad import or not. The question is not fully answered, and we are not prepared to dogmatise. General experience, however, leads us to believe that the reaction may indicate a bad prognosis in this manner, because it may be due to extension of the mischief before this gives rise to any definite clinical signs. Zoon states that the test is specific as applied by his technique, is of great diagnostic value in cases of complicated gonorrhœa in the male, adnexal inflammations in the female and in cases of arthritis. He says that the test is of no value in the diagnosis of vulvovaginitis in children, a statement with which the authors are inclined to agree, though their experience in this class of case has been very limited. As a result of specific study this author is able to state that syphilitic infection does not lead to fixation of complement in this test ; this has also been our general experience and has been specifically studied in the work of one of us (J. O. O.), already referred to.

It is well known that gonococci have been cultivated from the blood stream in the early stages of gonorrhœa. Some believe the infection to be commonly a bloodstream one. That such infection if frequent must be minimal and transitory is evidenced by the high percentage of negative complement fixation results obtained in the early stages of the disease. Any appreciable bloodstream infection would surely provoke a greater antibody response.

Regarding the disappearance of a positive reaction, we have already expressed the opinion that we do not regard the persistence of a positive reaction as absolute proof of a failure to cure, though we would qualify such a state-

ment by pointing out that before neglecting a persistent reaction as being of no import we would repeat the tests at an interval of a few weeks in order to ascertain if the reaction has diminished in strength. As we have already shown, other workers believe that with cure the reaction very rapidly, that is to say, within a few weeks, falls to the negative. In our experience such a course is not usual, and we have many cases which clinically and bacteriologically are to be considered as cured, and which even subsequent history likewise fails to incriminate, which have provided very slow falling off in the strength of the reaction over a period of many months and up to one year.

If then the reaction has once become strongly positive, we consider the best indication of cure from this point of view to be the gradual, though perhaps slow, diminution in its strength. Cases only weakly positive may become negative more quickly, often within three months.

The details of a few actual cases will illustrate many of the points we have raised :—

Case No. 1.—Male with synovitis of both knees. Original infection 12.4.27. Tested 3.5.27 gave a $+ \pm$ result. Apparent cure was effected by 6.10.27, yet the test on 6.3.28 (*i.e.,* five months after " cure ") gave a $\pm$ result.

Case No. 2.—Gonorrhœa in a male. No complications. 10.7.25 gave a $++$ result, on 19.4.26 the reaction was $+$, cure apparently took place by 31.12.26, yet six months later on, 14.6.27, the test still showed $\pm$.

Case No. 3.—Case of simple gonorrhœa in a male, the test on 3.6.25 gave $++$ result. Apparent cure had taken place by 6.8.25, yet the test had fallen to only $+$ by 30.4.26.

Case No. 4.—Case of gonorrhœa in a female, several months' duration, gonococci present in cervical discharge, reaction $+\pm$. After nine months' treatment, during which all trace of infection bacteriologically was lost, the reaction fell to $\pm$, and a further four months was required to bring the result down to the normal negative level.

Case No. 5.—Gonorrhœa in a female. History suggested infection at least two years previously. Gonococci present in cervical discharge. No complication. Reaction $++$. During two years' treatment the reaction

remained $++$ throughout, yet for the last eight months of the time no gonococci could be demonstrated, and clinically the patient appeared to be cured. A further five months' interval resulted in a result of $\pm$ being obtained, and again after another six months' interval the result was $\pm$, whilst after a final five months the result became negative.

Case No. 6.—Gonorrhœa in a female. Duration two years. Bartholin abscess one year previously. Reaction $+\pm$; twenty-eight months' interval with treatment intermittently led to a result of $+$ (*i.e.*, no marked drop) ; gonococci were still present, and vaccine together with milk therapy was commenced. Five months later the result was $++$ and gonococci were still present in the discharges. Two months later still the reaction was $++$, but at the end of that period gonococci could no longer be found. After a further twelve months' interval (during which apparent cure had taken place), the reaction had fallen to $\pm$. A further period of two and a half years was required before a cleanly negative result was obtained. Throughout this time there was no question of reinfection or continued infection.

Case No. 7.—Case of gonorrhœa in a female with a history of six weeks or less, reaction negative. During five months' treatment the reaction rose to $\pm$, gonococci being present throughout that time. The patient then defaulted for eight months, and the reaction was found to be $++$ at the end of that period. Three months' treatment followed, but the reaction remained $++$, gonococci being found on every occasion that examinations were made. Further default then occurred, and the reaction was then found to be $++$. Again the patient was treated for three months and again she defauted for ten months, the reaction remaining $++$. A further six months' treatment was given, gonococci were present throughout, and the reaction was then found to be $+\pm$. During the next five months' treatment gonococci could not be found constantly, although it was apparent that cure was not complete. The reaction meanwhile was $\pm$. Once more the patient defaulted for seven months, and on her return the result of the test was $+\pm$, and gonococci were again found quite easily and constantly. After a further period of intensive treatment lasting five months, cure became apparent, both clinically and bacteriologically, and at

the end of the period the test gave a result equivalent to negative. Watched for a further period of several months this patient showed no sign of relapse.

Case No. 8.—Arthritis of left ankle. Reaction $+\pm$. History of gonorrhœa twenty years previously. No present evidence of the disease.

Case No. 9.—Arthritis of right knee and left ankle. No gonococci found. Reaction $++$. History of gonorrhœa twelve years before.

Case No. 10.—Arthritis left ankle. Reaction $++$. History of gonorrhœa fifteen years before. Only present evidence of disease is some pus in the prostatic fluid.

Case No. 11.—Severe gonorrhœal rheumatism five years previously. Reaction $+\pm$. Other examinations negative.

Case No. 12.—Gonorrhœal arthritis. Reaction $++$ 27.4.26. Cured 26.10.26. Reaction negative 22.2.28.

Case No. 13.—Severe epididymitis for three days. Test negative. Inquiry elicited the fact that a blow from a cricket ball immediately preceded the attack.

Case No. 14.—Gonorrhœa in a female, several years' duration. Reaction $++$. Treated for five years without success, the reaction always remaining $++$ and gonococci being found at intervals throughout.

Case No. 15.—Gonorrhœa in a female, several months' duration. Reaction $+$; under treatment the patient developed Bartholinitis and the result was afterwards $++$. Treated for seven months, during which cure almost certainly took place and the reaction became negative. No subsequent relapse whilst under observation for one year.

Of these cases Nos. 1, 2, 3, 4, 5 and 6 all illustrate how slow may be the fall in the complement-fixing antibodies to the normal level. Case No. 7 illustrates the value of the test in long-standing cases, as a strongly positive result persisted throughout with infection. Cases Nos. 8, 9, 10 and 11 all show positive results in cases of arthritis in which latent infection is probable, and should be contrasted with Case No. 12, which shows that such a case will again give a negative result if cure really occurs.

Case No. 13 indicates how helpful the test may be in differential diagnosis, especially, as in the present instance, where a patient is not helpful.

Case No. 14 is one of continued infection in a female,

and shows that the test may well remain positive for an apparently indefinite period if cure does not occur. Finally, Case No. 15 illustrates a fairly rapid fall to negative after cure, even though a complication has been present.

CONCLUSIONS

(1) The very low percentage (0·6 per cent.) of false positive results appears to show that the test is remarkably specific.

(2) In all but the very early cases a high percentage of positive results is obtained, ranging from 50 per cent. and upwards, and reaching almost 100 per cent. in the cases of gonorrhœa with complications.

(3) Generally speaking, a positive result is not to be expected during the first ten days of the disease unless complications have occurred ; when an early positive result is obtained this is probably due to extension of the infection to the posterior urethra in the male or to the cervix uteri in the female.

(4) The authors are at variance with other workers with regard to the disappearance of the positive reaction with cure, since in their experience, following clinical cure, the change from positive to negative occupies in many cases a relatively long period.

(5) A positive result is almost diagnostic in any stage of the disease. A negative result is of similar significance to a negative Wassermann reaction ; it may occur in almost any stage of the disease and is of limited value except in the differential diagnosis of complications, such as arthritis and epididymitis.

(6) In tests of cure a negative reaction is of particular value if the case has previously given a positive reaction ; it has already been stated that a positive result may be retained for a varying time following clinical cure.

(7) Vaccines (more particularly stock ones) appear to have but little effect on the result of the reaction when used in the doses commonly employed, but it would appear that an autogenous vaccine is much more liable to influence the result.

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